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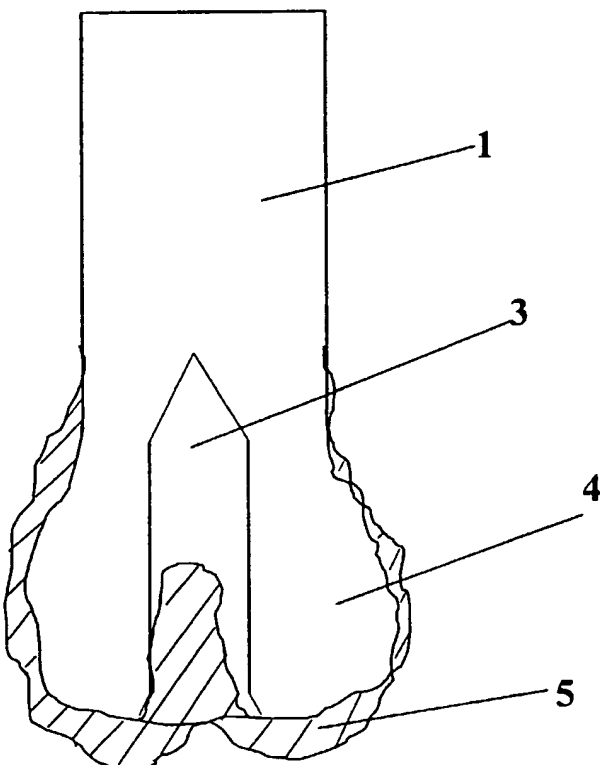
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(54) Title: SCAFFOLDS FOR TISSUE ENGINEERED HAIR



(57) Abstract: Porous, bioabsorbable scaffolds for tis-  
sue engineering of human hair follicles, methods for their  
manufacture and methods of their use in creating new  
hair.

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## SCAFFOLDS FOR TISSUE ENGINEERED HAIR

## BACKGROUND OF THE INVENTION

Male pattern baldness is a common condition that is often treated by hair transplant  
5 surgery. In this procedure hair follicles from areas of the scalp that are not within the  
baldness pattern are excised and re-implanted to create the illusion of a fuller head of hair.  
In fact, no new hair is created by this procedure, which is limited by the number of  
follicles that can be harvested for re-distribution. Thus, there is a great need, satisfied by  
the present invention, for a means for stimulating the growth of multiple new hair follicles  
10 in the scalp of an individual.

## BRIEF SUMMARY OF THE INVENTION

This invention relates to new bioabsorbable scaffolds that are useful for the tissue  
engineering of new hair follicles and to methods for their manufacture and to methods of  
15 their use in creating new hair. More specifically it relates to new and useful bioabsorbable  
porous structures that have the correct architecture to facilitate culturing of the appropriate  
follicle progenitor cells and their development into normal, functional, hair-producing  
follicles. The invention also relates to methods of making and using bioabsorbable  
scaffolds to implant and grow new hair follicles *in vitro* and *in vivo*.

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## BRIEF DESCRIPTION OF THE DRAWING(S)

Figure 1 is a cross-sectional view of form 1, with porous polymer precursor 5  
coating the distal end 4 of form 1.

Figure 2 is a cross-sectional view of porous scaffold 8 with a layer of follicle  
25 progenitor cells 9 lining the inner surface 10 of the porous scaffold.

Figure 3 is a side, three-dimensional view of a non-woven web 12 of bioabsorbable  
porous polymer material.

Figure 4 is a cross-sectional side view of a mold for forming a porous scaffold,  
comprising a top plate 14 having a bottom side 15 with forms 16 protruding from the  
30 bottom side of the top plate, and a bottom plate 17 with a top side 18 defining wells 19  
designed to receive the forms 16 of the top plate 14, when aligned therewith.

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Figure 5 is a top, slightly raised view of the non-woven web 12' after having been pressed and heated in the mold of Figure 4, forming depressions 20 in the non-woven web.

Figure 6 is a cross-sectional view of a set of scaffolds 10', after transfer to wells 22 of a cell culture dish 23.

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#### DETAILED DESCRIPTION OF THE INVENTION

In one aspect, the present invention comprises porous bioabsorbable scaffolds that are in the approximate shape of the normal hair follicle bulb, and designed to promote the formation of a hair follicle when seeded with hair follicle cells and implanted intradermally into a living host.

The scaffolds of the present invention are preferably comprised of a bioabsorbable polymer, selected from any of a wide variety of synthetic and natural polymers that are commonly used in clinical practice and in biomedical research. The scaffolds are more preferably comprised of a polymer selected from the group consisting of poly(lactic acid), poly(glycolic acid), poly(trimethylene carbonate), poly(amino acid)s, tyrosine-derived poly(carbonate)s, poly(carbonate)s, poly(caprolactone), poly(para-dioxanone), poly(ester)s, poly(ester-amide)s, poly(anhydride)s, poly(ortho ester)s, poly(amino acid)s, collagen, gelatin, serum albumin, proteins, carbohydrates, poly(ethylene glycol)s, poly(propylene glycol)s, poly(acrylate ester)s, poly(methacrylate ester)s, poly(vinyl alcohol), and copolymers, blends and mixtures of said polymers.

When the scaffold is comprised of a synthetic polymer, it is preferably a synthetic polymer formed from any one or combination of the following monomers: L-lactide, *d,l*-lactide, glycolide, trimethylene carbonate, caprolactone, and *para*-dioxanone. Other preferred synthetic polymers for use in making the scaffold of the present invention include poly(ethylene glycol), poly(vinyl alcohol), poly(acrylic acid) and other water soluble polymers that have been crosslinked with degradable linkages and any bioabsorbable hydrogel that has been modified to support cell attachment.

When the scaffold is comprised of a crosslinked or otherwise insoluble or insolubilized naturally occurring polymer, it is preferably a polymer selected from the group consisting of hyaluronic acid, human serum albumin, collagen, gelatin, cellulose derivatives, starch, dextrin, chitosan, and other proteins, glycoproteins, lipoproteins, polysaccharides, and biopolymers.

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A preferred scaffold of the present invention has an inner surface which is preferably in the shape of the outer surface of a hair root or bulb. The inner surface of the scaffold is preferably porous. The porosity of the scaffold is preferably sufficient to enable hair follicle cells to be adsorbed by the inner surface of the scaffold when placed  
5 into contact therewith, e.g., in a cell culture solution.

Methods of making the porous bioabsorbable scaffolds of the present invention are disclosed herein, below. Such methods include procedures for creating porosity in bioabsorbable materials and procedures for molding, shaping, or sculpting said porous scaffolds into the desired configuration. The present invention is not limited to scaffolds  
10 produced according to the specific methods disclosed herein, below, as it is contemplated that the scaffolds could be made using variations of the disclosed methods, or by adapting known means used to manufacture porous polymers.

Any one of a number of different means are suitable for creating the porosity of the scaffolds of the present invention. A preferred method for creating porosity involves the  
15 use of "blowing agents". These are chemical additives that decompose at known temperatures with the liberation of gases that cause foaming in the molten polymer and porosity in the resultant cooled material. A number of useful blowing agents are commercially available under the trade name of Celogen<sup>TM</sup> (Uniroyal Chemical Co.). One example of a traditional blowing agent is azodicarbonamide. Another blowing agent that  
20 may be especially useful in the present invention due to its compatibility with bioabsorbable polymers is urea dicarboxylic acid anhydride, described in U.S. Patent 4,104,195, the teachings of which are incorporated herein. The use of blowing agents can produce both open cell and closed cell foams. In the present invention open cells are desired and closed cells are to be avoided. Thus the conditions used in the manufacture of  
25 the porous coating are preferably optimized to achieve an open cell structure known as "reticulated" foam. The porosity of the scaffold is preferably due to interconnected pores in the size range of 0.1 to 1,000 microns, more preferably in the size range of 1 to 500 microns.

In an alternative embodiment, the porosity of the scaffold is due to the fact that the  
30 scaffold has a fibrous structure. When the scaffold has a fibrous structure, the fibers are preferably bonded together. The fibers of such a preferred structure are more preferably

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comprised of a core and sheath structure, said sheath being lower melting than said core, and bonded together by means of inter-fiber welds in the sheaths at points of contact.

One preferred method of making a scaffold of the present invention, (hereinafter, "the dissolution method") comprises the following steps:

- 5 1. Provide a bioabsorbable polymer that is soluble in a solvent (solvent A).
2. Provide a form in the shape of the desired scaffold made of a material that is soluble in a different solvent (solvent B) and that is substantially insoluble in solvent A.
3. Coat the form with particles of a pore-forming substance that is also soluble in solvent B or in a third solvent (solvent C).
- 10 4. Dissolve the bioabsorbable polymer in solvent A and apply the resultant solution to the particle-covered form.
5. Remove solvent A by evaporation or other suitable means.
6. Use solvent B to dissolve said form and said particles.
7. Remove solvent B from the finished porous scaffold.
- 15 8. If required, use solvent C additionally to remove said particles.

The dissolution method is illustrated in Figure 1, which shows form 1 in cross-section with distal end 4 coated with porous polymer precursor 5, a mixture of particles of the pore forming substance and a solution of the polymer dissolved in solvent A. Note that  
20 the distal end 4 of form 1 is bulbous in shape, and includes a cavity 3 sufficiently large enough to enable some of the porous polymer precursor 5 to enter the cavity. Once a porous polymer has been formed from the porous polymer precursor 5 of Figure 1, as described above, and the form has been dissolved, the resulting porous scaffold can be seeded with hair follicle progenitor cells and used as described below.

25 Figure 2 is a cross-sectional view that shows the porous scaffold 8 obtained by dissolving and washing away the pore forming substance and form, as described above, leaving only the bioabsorbable polymer. The scaffold 8 is shown with follicle progenitor cells 9 seeded on to the inner surface 10 of the scaffold, having taken the shape of the scaffold. The structure shown in Figure 2 could be directly implanted into the dermis to  
30 promote the growth of a new single hair fiber from the tissue engineered follicle. However, to better ensure that the transplanted engineered follicle matures, the structure shown in Figure 2 is more preferably cultured with additional cells prior to implantation.

Examples of materials that can be used to create the form and solvents that can be selected for use as solvent B in the dissolution method, described above, include the following combinations: poly(ethylene oxide) and water; paraffin wax and hexane; and polystyrene and acetone. The pore forming substance and form material must be selected from those substances that have low solubility in solvent A, used to introduce the bioabsorbable polymer into the structure. These choices are further exemplified in Table 1 below where the following abbreviations have been used: PLGA is a copolymer of lactic and glycolic acids and PEO is poly(ethylene oxide).

Table 1.

Bioabsorbable polymer	Form material	Pore forming substance	Solvent A	Solvent B	Solvent C
PLGA	PEO	Sodium chloride	Acetone	Water	Water
PLGA	Wax	Glucose	Acetone	Hexane	Water
Collagen	Wax	Polystyrene	Water	Hexane	Acetone

A modification of the dissolution method is exemplified by reversing the sequence of steps of creating the desired structure followed by seeding with progenitor cells. Thus a porous scaffold structure can first be formed in the shape of a disc, for example by adding a solution of polymer in an organic solvent to appropriately sized salt particles in a cylindrical container followed by evaporation of said solvent and removal of the salt by dissolving and rinsing with water.

The resultant highly porous scaffold can then be sterilized and seeded with micro-dissected human dermal papilla or other suitable source of follicle progenitor cells and cultured *in vitro* until the entire porous structure is populated with cultured cells. This tissue-engineered construct then can be cut up into a large number of fragments, each about the size of a normal human dermal papilla. These irregular shaped fragments can be suspended in culture media and cultured further until the desired smooth surfaced structure is obtained. These tissue engineered dermal papilla can be implanted or injected into the skin to initiate the process of follicle neogenesis for hair restoration.

Alternatively, the porous scaffolds of the present invention can also be made according to the following method (hereinafter, the "pressed mold method"), comprising the following steps:

1. Provide a thin, non-woven web of bioabsorbable fibers.

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2. Place said web in a two-part mold that has cavities in one part and mating forms in the other part, said cavities and forms providing the desired shape and dimensions of the desired scaffolds.

3. Close the mold and apply sufficient heat and pressure to form the web into the desired porous structure.

4. Remove the web from the mold and die-cut the molded scaffolds from the web.

The non-woven web preferably comprises either fibers that have a core/sheath structure in which the core of the fiber has a higher melting temperature than the sheath, or fibers without such a structure. The fibers in the non-woven web are preferably felted, sintered, or bonded with the use of a solvent or a second polymer dissolved in a solvent.

Figures 3-6 illustrate application of the mold method to produce scaffolds of the present invention. Figure 3 shows a drawing of a non-woven web 12 of bioabsorbable fibers. Figure 4 shows a two-part mold, comprising a top plate 14 and a bottom plate 17. The top plate features a bottom surface 15 with an array of forms 16 protruding therefrom. The bottom plate features a top surface 18 with depressions 19 therein to receive said forms. Figure 5 is a slightly raised, angled view of the non-woven web 12' after it has been compressed and heated in the mold of Figure 4, thereby creating depressions 20 that are molded into the desired shape for scaffolds for tissue engineered hair. Figure 6 shows molded scaffolds 8' after they have been cut from the depressions 20 in the non-woven web 12' in Figure 5 by means of a die cutter, and transferred to wells 22 of culture dish 23. The wells 22 are designed to receive scaffolds 8 or 8', culture medium, and cells.

The mold production process described immediately above is particularly well suited for scale-up and mass production. For example, the two-part mold shown in Figure 4 could be produced in the form of two cylinders rather than two flat plates. The web, in the form of a continuous ribbon, could be embossed with the desired pattern as it is compressed and heated during passage between the two counter-rotating rolls. Die cutters also could be mounded in a rotating cylinder to cut out the scaffolds or the cutter could be an added feature of the embossing rolls. This automated assembly line process would continue with the scaffolds being deposited into cell culture wells that are pre-formed in polyester film. The scaffold- loaded film could be cut and packaged into trays, placed in additional packaging and sterilized.



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Other methods of creating porous scaffolds from bioabsorbable materials also can be used in practice of the present invention. Methods such as emulsion freeze-drying, expansion in high pressure gas, 3D printing, and phase separation techniques are discussed in an article by Y.S. Nam and T.G.Park, "Porous biodegradable polymeric scaffolds prepared by thermally induced phase separation", *The Journal of Biomedical Materials Research*, Oct. 1999, vol. 47, no. 1, pages 8-17, the teachings of which are incorporated herein.

A preferred embodiment of the above-mentioned phase separation technique uses poly(*d,l*-lactide-co-glycolide) (PLGA) as the structural polymer and poly(ethylene glycol) (PEG) as the porogen. Thus a mixture of PLGA and PEG can be dissolved in dichloromethane to give a clear solution, which is then applied to a scaffold form made from a material that is not soluble in dichloromethane, but preferably soluble in water, for example sugar. Upon evaporation of the dichloromethane, the PEG phase separates from the PLGA by crystallization. If the form is made of sugar, then soaking in water dissolves out the PEG as well as the form to leave the resultant desired porous PLGA scaffold.

Scaffolds of the present invention can be used to engineer new hair follicles in a number of ways. In general, the appropriate cells can be seeded on the scaffold and either implanted immediately into the scalp or allowed to multiply in culture on the scaffold prior to implantation. The implantation procedure can be the same technique that hair transplant surgeons currently use to implant single follicles or "mini-grafts". For example, a laser can be used to bore a small hole in the scalp to precisely the desired depth and the cell-seeded scaffold can simply be planted in the hole. As these implanted cells grow they orchestrate the neo-genesis of a new hair follicle. The bioabsorbable scaffold then degrades and is eliminated from the site as the implant matures into a normal, hair-producing follicle.

The cells used to seed the scaffold can be taken from follicles biopsied from the patient or from organ donor follicles. This later option is known to be feasible due to recent research results. Follicle progenitor cells from a human donor were successfully transplanted into an unrelated human recipient where they initiated the formation of new follicles that grew hair. This finding, entitled "Trans-gender induction of hair follicles", was reported by A.M. Reynolds, C. Lawrence, P.B. Caerhalmi-Friedman, A.M. Christiano and C.A.B. Jahoda in *Nature*, 402, 33-34, November 4, 1999, the teachings of which are

incorporated by reference herein. A distinct advantage of the present invention is the ability to multiply the cells in culture before seeding them on the scaffold implants. This both maximizes the number of scaffolds that can be seeded from each harvested follicle and minimizes the labor of dissecting follicles to obtain the desired progenitor cells.

5

## EXAMPLES

### Example 1:

Poly(ethylene oxide) (hereinafter, "PEO") 100,000 molecular weight purchased from Aldrich Chemical Co. (Milwaukee, WI 53201), was melt extruded into a 1.0 mm diameter filament and cut into 2 cm lengths. One of the PEO filaments was dipped into water, hydrating the surface and making it sticky. This was then dipped into sodium chloride crystals that had been ground into fine particles in an electric coffee bean grinder. Excess salt was shaken off and the coating was allowed to dry. A 10% (w/v) solution of poly(*d,l*-lactide-*co*-50%-glycolide) (PLGA) (Resomer RG504, Boehringer Ingelheim, Germany) in acetone (Aldrich Chemical Co.) was dripped onto the salt encrusted PEO filament and the excess solution was allowed to run off. Additional powdered salt was sprinkled onto the surface until it was completely covered. After the acetone evaporated, the coated PEO filament was placed in water until all of the salt and PEO dissolved, leaving a hollow filament of porous PLGA which was removed, flattened, and cut into thin strips with a sharp blade. The strips were rolled between finger and thumb and cut into 2 mm lengths. The hollow filament of porous PLGA produced as described above was used to make a form for a scaffold of the present invention, as follows.

Scaffolds of the present invention were produced as follows. A 0.3 mm diameter concentric hole was made in the end of another PEO filament by pressing a heated needle about 2 mm into the PEO. This caused molten PEO to build up around the sides of the filament. Upon cooling, the needle was removed. The hole was then filled with one of the above rolled strips of porous PLGA. The end of the resulting PEO filament was then coated as described above with salt and PLGA solution. Upon evaporation of the acetone and dissolving all of the salt and PEO in water and drying, the desired porous bioabsorbable polymer scaffold was obtained.

30

## Example 2:

Human hair follicles are dissected to obtain the dermal papilla, which are transferred to a culture flask containing culture media. After several weeks in culture, the dermal papilla cells multiply and grow over the surface of the cell culture flask. These  
5 cells are detached from the flask by treatment with an enzyme and concentrated by centrifugation. The cells are then transferred, after re-suspension, by pipette into the scaffolds of Example 1 and the cell-seeded scaffolds placed in a culture flask with media for several days to allow the cells to adhere to the surfaces of the scaffolds. Culturing of the cell-seeded scaffolds is then continued in another flask of media with gentle stirring  
10 until the scaffolds are fully populated with cells.

## Example 3:

Scaffolds seeded as described in Example 2 are implanted into the scalp of a human experiencing hair loss. Over time, as new hair follicles are created, new hairs grow  
15 from the implants, and the scaffolds bioabsorb.

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## CLAIMS

1. A porous, bioabsorbable scaffold for tissue engineering of human hair follicles.
- 5 2. A scaffold of claim 1 in which said bioabsorbable material is comprised of a polymer synthesized from one or more of the following monomers: L-lactide, *d,l*-lactide, glycolide, trimethylene carbonate, caprolactone, and *para*-dioxanone.
3. A bioabsorbable material of claim 2 in which said polymer is a copolymer of *d,l*-  
10 lactide and glycolide.
4. A scaffold of claim 1 in which said bioabsorbable material is collagen.
5. A scaffold of claim 1 in which said porosity is due to interconnected pores in the  
15 size range of 0.1 to 1,000 microns.
6. A scaffold of claim 5 in which said pores are in the size range of 1 to 500 microns.
7. A scaffold of claim 1 in which said porosity is due to a scaffold with a fibrous  
20 structure.
8. A scaffold of claim 7 in which said fibrous structure is comprised of fibers that are bonded together.
- 25 9. A scaffold of claim 8 in which said fibers are comprised of a core and sheath structure, said sheath being lower melting than said core, and are bonded together by means of inter-fiber welds in the sheaths at points of contact.
10. A method of making a scaffold of claim 1 comprising the steps of,  
30 a. Provide a bioabsorbable polymer that is soluble in a solvent (solvent A).  
b. Provide a form in the shape of the desired scaffold made of a material that is soluble in a different solvent (solvent B) and that is substantially insoluble in solvent A.

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- c. Coat the form with particles of a substance (the pore-forming substance) that is also soluble in solvent B or in a third solvent (solvent C).
  - d. Dissolve the bioabsorbable polymer in solvent A and apply the resultant solution to the particle-covered form.
  - 5 e. Remove solvent A by evaporation or other suitable means.
  - f. Use solvent B to dissolve said form and said particles.
  - g. Remove solvent B from the finished porous scaffold.
  - h. If required, use solvent C additionally to remove said particles.
- 10 11. A method of making a scaffold of claim 1 comprising the steps of,
- a. Providing a bioabsorbable polymer that is soluble in a first solvent and substantially insoluble in a second solvent.
  - b. Providing a form in the shape of the desired scaffold made of a material that is soluble in the second solvent and substantially insoluble in the first solvent.
  - 15 c. Providing a pore-forming substance that is soluble in both first and second solvents.
  - d. Coating the form with a solution of bioabsorbable polymer and pore-forming substance dissolved in the first solvent.
  - e. Removing the first solvent and causing the pore forming substance to phase  
20 separate from the bioabsorbable polymer.
  - f. Using the second solvent to dissolve said form and said pore forming substance.
  - g. Removing the second solvent from the finished porous scaffold.
- 25 12. A method of making a scaffold of claim 11 in which said bioabsorbable polymer is a copolymer of d,l-lactide and glycolide, said pore forming substance is poly(ethylene glycol), said form is made of sugar the first solvent is dichloromethane and the second solvent is water.
- 30 13. A method of making a scaffold of claim 1 comprising the steps of,
- a. Providing a thin, non-woven web of bioabsorbable fibers.
  - b. Placing said web in a two-part mold that has cavities in one part and mating

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forms in the other part, said cavities and forms providing the desired shape and dimensions of the desired scaffolds.

c. Closing the mold and applying sufficient heat and pressure to form the web into the desired porous structure.

5 d. Removing the web from the mold and die-cut the molded scaffolds from the web.

14. A method of creating new hair comprising the steps of,

a. Providing scaffolds of claim 1.

10 b. Seeding hair follicle progenitor cells on said scaffolds.

c. Implanting scaffolds of step 2 into skin where the growth of new hair is desired.

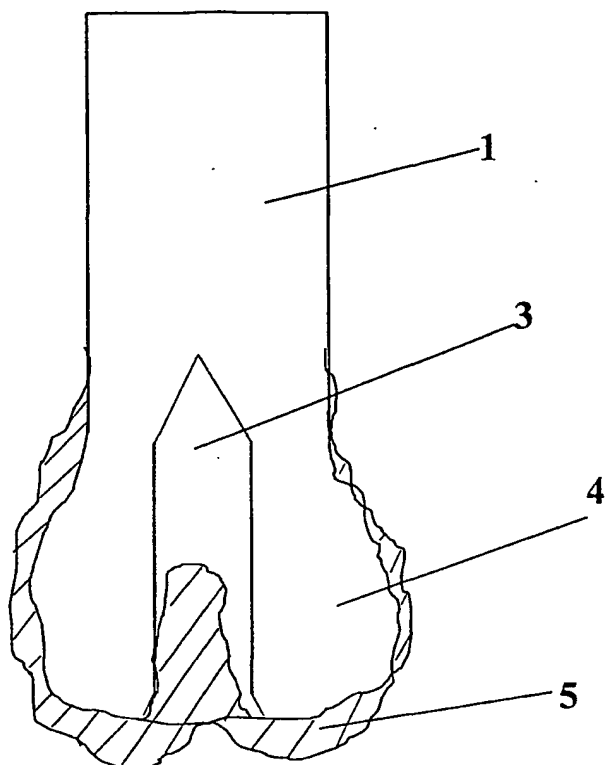
15 15. The method of creating new hair of claim 14 in which said progenitor cells used in step 2 are obtained from substructures within normal hair follicles that are known to contain such cells including the dermal papilla, the dermal sheath and the bulge area.

16. The method of creating new hair of claim 15 in which said progenitor cells are multiplied in culture prior to seeding on the scaffolds of claim 1.

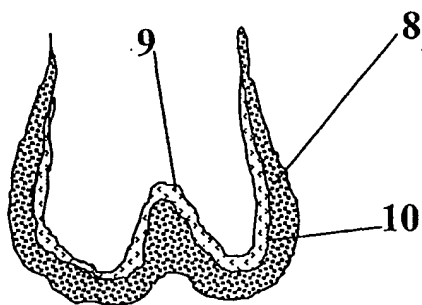
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17. The method of creating new hair of claim 14 in which the method of implantation of said scaffolds in step 3 is substantially equivalent to the procedure currently used by hair transplant surgeons to implant single hair grafts into the scalp.

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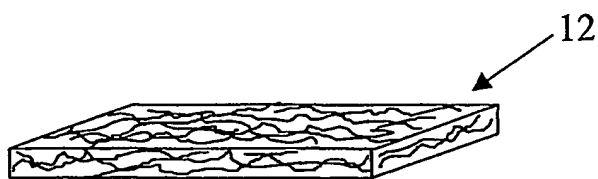


**FIG. 1**

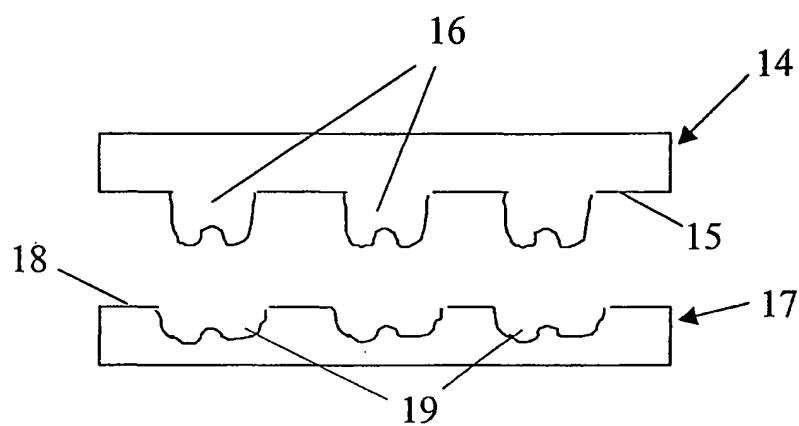


**FIG. 2**

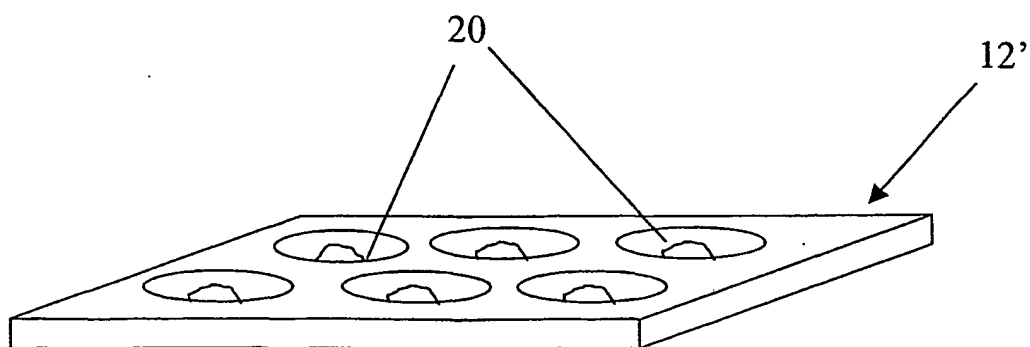
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**FIG. 3**



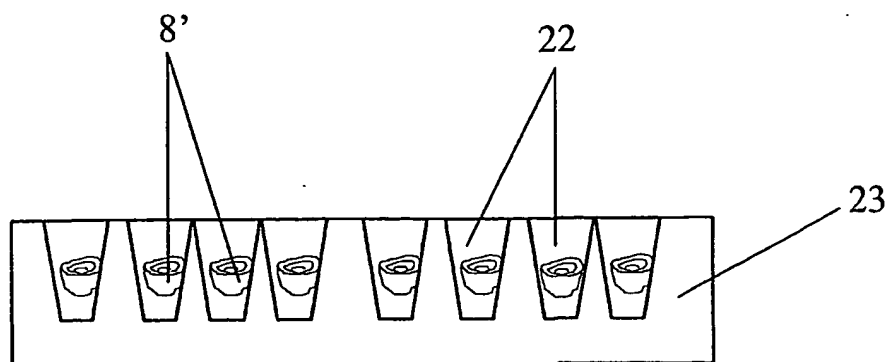
**FIG. 4**



**FIG. 5**



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**FIG. 6**

## INTERNATIONAL SEARCH REPORT

International Application No

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## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61L27/56 A61L27/18 A61L27/26 A61L27/38 A61F2/10  
C12N5/06

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 002 859 A (ISOTIS B V) 24 May 2000 (2000-05-24) column 4, line 22 - line 45; claims; example ---	1,2,7
P,X	WO 00 62829 A (LHOMMEAU CHRISTELLE M ; KOHN JOACHIM B (US); LEVENE HOWARD B (US);) 26 October 2000 (2000-10-26) claims; examples ---	1-3,5-7, 10-12
P,Y	WO 00 45736 A (BIOAMIDE INC ; BARROWS THOMAS H (US)) 10 August 2000 (2000-08-10) claims; examples 1-3 ---	1-17
Y	US 5 514 378 A (CIMA LINDA G ET AL) 7 May 1996 (1996-05-07) claims; examples 1-4 ---	1-17
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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 502 092 A (SUSZKO PAUL R ET AL) 26 March 1996 (1996-03-26) claims; examples ---	1-17
A	WO 99 34750 A (BIOAMIDE INC ;BARROWS THOMAS H (US)) 15 July 1999 (1999-07-15) claims; examples ---	1-17
A	WO 00 29553 A (ORGANOGENESIS INC ;RONFARD VINCENT (US); MURPHY MICHAEL P (US)) 25 May 2000 (2000-05-25) claims ---	1-17
A	WO 98 44027 A (SHEA LONNIE ;UNIV MICHIGAN (US); HARRIS LEATRESE (US); MOONEY DAVI) 8 October 1998 (1998-10-08) claims -----	1-17

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/24671

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 1002859	A	24-05-2000	EP 1002859 A1	24-05-2000
WO 0062829	A	26-10-2000	WO 0062829 A1 AU 3566399 A	26-10-2000 02-11-2000
WO 0045736	A	10-08-2000	AU 3594100 A EP 1156756 A1 WO 0045736 A1	25-08-2000 28-11-2001 10-08-2000
US 5514378	A	07-05-1996	NONE	
US 5502092	A	26-03-1996	EP 0744969 A1 JP 9509082 T WO 9522360 A1 US 5856367 A	04-12-1996 16-09-1997 24-08-1995 05-01-1999
WO 9934750	A	15-07-1999	AU 734539 B2 AU 2106499 A EP 1045677 A1 WO 9934750 A1	14-06-2001 26-07-1999 25-10-2000 15-07-1999
WO 0029553	A	25-05-2000	AU 1917100 A BR 9915476 A EP 1131410 A1 WO 0029553 A1	05-06-2000 02-01-2002 12-09-2001 25-05-2000
WO 9844027	A	08-10-1998	AU 736102 B2 AU 6786098 A EP 0991705 A1 WO 9844027 A1 US 6281256 B1	26-07-2001 22-10-1998 12-04-2000 08-10-1998 28-08-2001